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Genomic Analysis and Prediction within a US Public Collaborative Winter Wheat Regional Testing Nursery

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ABSTRACT The development of inexpensive, whole-genome profiling enables a transition to allele-based breeding using genomic prediction models. These models consider alleles shared between lines to predict phenotypes and select new lines based on estimated breeding values. This approach can leverage highly unbalanced datasets that are common to breeding programs. The Southern Regional Performance Nursery (SRPN) is a public nursery established by the USDA–ARS in 1931 to characterize performance and quality of near-release wheat (*Triticum aestivum* L.) varieties from breeding programs in the US Central Plains. New entries are submitted annually and can be re-entered only once. The trial is grown at >30 locations each year and lines are evaluated for grain yield, disease resistance, and agronomic traits. Overall genetic gain is measured across years by including common check cultivars for comparison. We have generated whole-genome profiles via genotyping-by-sequencing (GBS) for 939 SPRN entries dating back to 1992 to explore the potential use of the nursery as a genomic selection (GS) training population (TP). The GS prediction models across years (average *r* = 0.33) outperformed year-to-year phenotypic correlation for yield (*r* = 0.27) for a majority of the years evaluated, suggesting that genomic selection has the potential to outperform low heritability selection on yield in these highly variable environments. We also examined the predictability of programs using both program-specific and whole-set TPs. Generally, the predictability of a program was similar with both approaches. These results suggest that wheat breeding programs can collaboratively leverage the immense datasets that are generated from regional testing networks.

Abbreviations: BLUP, best linear unbiased predictor; BP, breeding population; GBS, genotyping-by-sequencing; GS, genomic selection; RPN, Regional Performance Nursery; SNP, Single nucleotide polymorphism; SRPN, Southern Regional Performance Nursery; TP, training population.

Core Ideas
- Genomic selection in wheat
- Regional testing nurseries
- Allele-based breeding

Plant breeding programs exert considerable effort evaluating new breeding lines across many locations to identify superior-performing candidates for release as new varieties. For this evaluation in wheat, collaborative regional testing networks have been developed in the United States to provide additional information to breeders on the broad performance of their lines.

The US cooperative regional performance testing program was established in 1931 by the USDA–ARS in partnership with university agricultural experiment stations to characterize performance, quality, disease resistance, and other agronomic traits of near-release wheat varieties from breeding programs in the US Central Plains (Graybosch, 2017a). In this network, the SRPN and the Northern Regional Performance Nursery for winter wheat were established and allow breeders to submit entries that are distributed for evaluation at >30 locations along with multiple, common, long-term check varieties.
Phenotypic data collected from the nurseries includes grain yield, test weight, plant height, lodging, and resistance to a variety of diseases. The regional performance nurseries have been used to regularly measure genetic gain over time (Schmidt and Worrall, 1983; Graybosch and Peterson, 2010, 2012), evaluate long-term wheat diversity (Cox and Worrall, 1987), and cluster experimental locations into production zones based on performance data (Peterson, 1992; Graybosch, 2017b).

Previous investigation of broad genotypic characteristics of the Regional Performance Nurseries (RPNs) has been limited because of the overall number of lines that have been tested, difficulty in obtaining a complete set of evaluated entries, and an inherent challenge in generating a sufficient amount of genotypic data for each entry. With the recent development of inexpensive, high-density genetic markers, whole-genome marker profiles can now be obtained for every experimental line, making possible new analyses that rely on large amounts of genomic data including diversity studies and genomic selection (Poland and Rife, 2012).

Genomic selection is a statistical approach that is used to predict phenotypes and select new lines in breeding programs based on the total allelic effects across the genome (Meuwissen et al., 2001). Breeding programs are investigating and using GS as a tool to shorten the breeding cycle (Heffner et al., 2009, 2010) and increase selection intensity (Cros et al., 2015; Battenfield et al., 2016). Genomic selection has two fundamental components: (i) a population that has been both phenotyped and genotyped that is used to train the prediction model and (ii) a population that has been only genotyped to which the model is then applied and the predictions used to select superior breeding lines (Heffner et al., 2009, 2010). Previous literature has assigned each of these two populations various designations (Rincent et al., 2012; Rutkoski et al., 2015; Isidro et al., 2015). Here we will refer to the two populations as the TP and the breeding population (BP), respectively.

Optimal design of the TP is a research topic of high interest to the breeding community as the phenotypic evaluation of the TP remains a time-consuming and expensive endeavor (Akdemir et al., 2015; Spindel et al., 2015; Isidro et al., 2015). There is still limited understanding of the characteristics that make up an ideal TP. However, two features have been promoted as compelling factors: size and degree of relatedness. As the number of lines used in the TP increase, there is a concurrent increase in the accuracy of the predictions (Zhong et al., 2009). However, there are generally diminishing returns, with reduced gains in accuracy as more lines are added to the TP (Asoro et al., 2011). Similarly, a TP and BP must be interrelated with common alleles and markers for suitable predictive ability. A TP that is more closely related to the BP often results in better prediction accuracy (Hayes et al., 2009; Long et al., 2011; Pszczola et al., 2012; Rutkoski et al., 2015). An inherent feature of plant breeding programs is the shifting of allele frequencies at each stage of breeding, ultimately limiting allelic diversity present in elite material to a subset of the total diversity present in the BP. While the most elite material is less representative of the entire allelic diversity of the program, it is the most extensively phenotyped. Elite testing nurseries, therefore, are often included in the TP and serve to give good estimates of the most favorable haplotypes.
The broad scope and design of the RPN makes it an ideal collection to investigate both factors affecting the TP since thousands of lines from different regional programs have been evaluated in this nursery, and it is extensively phenotyped across many locations. The simultaneous interrelatation and stratification of alleles between the regional breeding programs makes it possible to examine how relatedness factors into accuracy both across and within programs. A successful implementation of GS using the lines that have been evaluated in the RPN would allow plant breeders in the region to leverage this data to transition to allele-based breeding and to predict stable, broad adaptation. Prediction models that account for alleles shared between lines would make it possible to use the vast quantities of phenotypic data available from this nursery. To this end, we have generated whole-genome profiles via GBS for SRPN entries dating back to 1992. This genetic data was used to characterize the potential for the SRPN to serve as a TP for GS and evaluate prediction differences between breeding programs.

**MATERIALS AND METHODS**

**Plant Material**

A collection of 939 entries (691 unique lines) that were submitted to the SRPN from 1992 through 2015 was assembled and DNA was extracted from seedling leaf tissue using a BioSprint 96 DNA Plant Kit (Qiagen). The DNA was quantified in plates using PicoGreen and normalized to 20 ng L$^{-1}$.

**Library Construction and Data Processing**

Fourteen GBS libraries were prepared following the protocol detailed by Poland et al. (2012a). Briefly, DNA was digested with \(PstI\) and \(MspI\) and barcoded adapters were ligated to the ends of the fragments. Samples were then pooled at 192-plex, amplified, and sequenced on an Illumina HiSeq 2000. Single nucleotide polymorphisms (SNPs) were called using the approach of Poland et al. (2012b) using a population-based filter. The SNPs were filtered to have >5% minor allele frequency and at least 20% of the data present across samples. For subsequent genomic prediction, genotype data from missing SRPN entries for which seed was unavailable were imputed when the same breeding line had been evaluated in a different year (as a different entry). All sequence data is available from the NCBI Sequence Read Archive under SRP149777.

**Phenotypic Data**

Historical phenotypic data from 82,546 plots was compiled and a mixed linear model was used to calculate best linear unbiased predictors (BLUPs) for lines with random effects for entry, location, year, location-by-year interactions, and replication within location-by-year interactions using the lmer command from the lme4 package in R (Bates et al., 2014) (Supplemental Table S1). While the SRPN allows experimental varieties to be evaluated in the nursery for up to 2 yr, re-entries were treated separately within the individual year in which they were evaluated. In other words, a single experimental variety submitted by a breeder over two consecutive years was treated as two different entries in this study for calculating BLUPs. During the years evaluated in this study, 207 entries were submitted to the nursery for two consecutive years. These entries were used to calculate a phenotypic correlation for yield across years via a Pearson correlation of the performance of Year 1 with the performance of Year 2 for all entries. All historical entry and phenotypic data are available from the Dryad Digital Repository (https://doi.org/10.5061/dryad.q968v83).

**Genomic Prediction**

A realized additive relationship matrix (\(A\)) was constructed using the A.mat function in the rrBLUP package in R (Endelman, 2011). Markers were imputed using the EM algorithm and a maximum missing threshold of 0.8 was used. The kin.blup function in the rrBLUP package was then used to perform genomic prediction with \(K\) set to \(A\) (Endelman, 2011). Two separate TP schemes were evaluated. The first was a temporal-based TP constructed such that all lines tested in previous years were used as the TP for a given year resulting in a TP that increased in size by ~40 new lines each year. After running the predictions for all years, predictions were recalculated after excluding 2001 from the TPs due to nonrepresentative conditions caused by a stripe rust epidemic (Line, 2002).

The second approach examined the prediction accuracy of lines from a given breeding program using a TP consisting of either (i) all lines from all the programs or (ii) only lines from the same program. Both methods used a leave-one-out approach wherein a single line was removed from the group and the remaining lines were used as the TP to predict the performance of the missing line (Efron and Gong, 1983). Method 1 was performed across all entries by subsetsing the predicted values by breeding program and then calculating a Pearson correlation between the predicted values and BLUPs. Method 2 was performed using only entries from within each breeding program as the TP.

**RESULTS AND DISCUSSION**

**Genotyping**

To move from line-based breeding to allele-based breeding methods, whole-genome profiles were generated for all available entries from the SRPN and subsequently used to calculate a realized relationship matrix. In this study, we used GBS with an internal alignment-based pipeline to discover and genotype 53,672 SNPs with 2463 of these SNPs having >80% data present.

**Phenotypic Data Analysis**

Yield data from 82,546 plots, representing 670 unique location-year nurseries, was used in a mixed linear model to calculate a BLUP for each of 1003 SRPN entries,
after removing nonrepresentative, historical check cultivars. Most of the entries submitted to the SRPN are only tested for a single year, making absolute yield comparisons across all years impossible. However, 207 of the experimental lines submitted to the SRPN were evaluated twice in subsequent years, it was possible to use the performances (i.e., BLUP) of these lines from their first year and second year in the nursery to obtain an estimate of the year-to-year phenotypic accuracy in the nursery. The Pearson correlation for plot yield across years in these lines was moderately low at 0.27 \((p < 0.01, 95\% \text{ CI [0.143, 0.395]})\), likely due to large genotype \(\times\) environment interactions. This is to be expected because of the wide range and diversity of environments from which data is being generated and the high year-to-year variation common to the US Central Plains.

**Genomic Prediction Across Years**

A temporal-based TP was created that used data from all previous years to make predictions on the next year. Genomic prediction using this approach resulted in an average Pearson correlation between the calculated BLUPs and predicted values of 0.33 (Fig. 2). The correlations for 11 of the 23 predicted years were significant at \(p < 0.05\). This approach created a TP that increased in size with each subsequent prediction cycle. However, there was not an observed positive trend in prediction accuracy with the increased TP size likely caused by the large influence that the year of evaluation has on the yield of entries within the nursery and the low heritability of yield common to the region (Dawson et al., 2013; Rutkoski et al., 2015; Lado et al., 2016).

**For GS to be implemented into breeding programs, it needs to have similar accuracy to or surpass the selection methods being used by breeders, namely, phenotypic selection. To benchmark these GS predictions against phenotypic selection, we compared the genomic prediction accuracies with the phenotypic correlation of lines that were evaluated multiple times in the SRPN. Predictions were superior to the phenotypic correlation in 12 of the 23 predicted years and within or better than the 95\% confidence interval of the phenotypic correlation in all but two of the predicted years (Fig. 2). Notably, genomic predictions significantly outperformed the phenotypic correlation estimate in seven of the years evaluated. Further investigation into why the entries within these years were predicted so well relative to others is needed, as there was not a readily apparent explanation. We observed very poor (e.g., negative) prediction accuracy for the 2001 nursery. One potential explanation for the drastic decrease in predictive accuracy in 2001 was an epidemic of stripe rust resulting from a mild winter (Line, 2002).**

**Genomic Prediction Across Breeding Programs**

To determine if data from other breeding programs can be used for genomic prediction within a given breeding program, separate TPs consisting of all experimental lines (excluding the line being predicted) and lines specific to a given breeding program (excluding the line being predicted) were used to predict lines one at a time within a breeding program (Fig. 3). There is a trend in prediction accuracy that is independent of the approach used. Breeding programs that are relatively predictable with one...
method are also relatively predictable with the other. This implies that the potential for a breeding program to implement GS is likely to be founded on characteristics intrinsic to a given program and that, as tested here, GS may perform better in some breeding programs because of the diversity, effective population size, or pedigree relationships (Lorenz et al., 2011). One potential hypothesis for the differences in predictability between breeding programs may be the frequency or fixation of certain major-effect adaptation loci within each breeding program. However, we did not identify significant population structure to offer support for this claim (Supplemental Fig. S1).

CONCLUSIONS

Maintaining long-term, regional testing networks, as well as their seed stocks, can provide additional information for genetic improvement and ensure future crop production and food security. The potential to use existing historical datasets for new breeding approaches, like GS, is attractive since generating new phenotypes is both cost- and time-prohibitive and the sampling of many past years of environments has immense value. In this study, we examined and considered multiple approaches to implement GS using historical data from the US SRPN. Genomic predictions across the entire collection outperformed a year-to-year phenotypic correlation (i.e., phenotypic selection accuracy). However, these results were not consistent across breeding programs, with several programs showing reduced or no predictive ability. Our results indicate that there may be inherent characteristics of breeding programs such as germplasm base or target region that prohibit or constrain the use of information from other breeding programs and regional testing networks for genomic prediction as a tool for selection. With the increasing need to maximize genetic gain and accelerate delivery of improved high-yielding varieties, the use of historical data from coordinated testing networks can be a valuable addition to the genomic prediction models used by plant breeders.

Fig. 3. The prediction accuracies for individual breeding programs. Each row contains the name of the breeding program, the number of lines used in the analysis, the correlation ($r$) when using a training program comprised of all lines (left), and the correlation when using only lines originating from the same breeding program (right). Boxes are shaded based on correlations. Significance is indicated at the 0.05 (*), 0.01 (**), and 0.001 (***), levels.
Supplemental Information

Supplemental Fig. S1. Plot of the first and second Eigen vectors derived from the A matrix using the eigen function in R (R Development Core Team, 2017).

Supplemental Fig. S2. Dendrogram showing relationship of the wheat lines used in this study created using the gbs.dendro function in the gbs-r package in R (https://github.com/trife/gbs). Lines were grouped and colored based on originating breeding program.

Supplemental Table S1. Calculated BLUPs for each of the lines used in the analysis.

Conflict of Interest Disclosure

The authors declare that there is no conflict of interest.

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